

## OSMOSIS AND SWELLING OF GELATIN.

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Progress in the study of the physical and chemical properties of gelatin has been retarded because of the difficulty of preparing material of standardized purity. The swelling and osmosis experiments of previous investigators, including Ostwald,<sup>1</sup> Lillie,<sup>2</sup> Moore and Roaf,<sup>3</sup> Procter,<sup>4</sup> Procter and Wilson,<sup>5</sup> Loeb,<sup>6</sup> and others, have been conducted with gelatin containing ash. The presence of electrolytes, however, has a profound influence upon the osmosis and swelling of gelatin as well as upon its precipitation by alcohol and other properties.

Having succeeded in preparing electrolyte-free gelatin from material standardized by its optical rotation,<sup>7</sup> the writer has studied the osmosis and swelling of its sols and gels with and without electrolytes. An attempt to correlate these two closely dependent sets of phenomena has been made.

**Ash-free (Electrolyte-free) Gelatin.**

Paal<sup>8</sup> made boiling-point determinations on gelatin stated to contain 0.07% ash, but failed to state how his product was obtained, thus leaving uncertain the nature of his preparation.

Dhère and Gorgolewski<sup>9</sup> claimed to have obtained "demineralized" gelatin by dialysis of gelatin sheets for 1½ to 3 months, using ether as a preservative. They also found that on freezing out the water from a 0.5% solution of gelatin an almost ash-free gelatin was obtained. Exhaustively dialyzed gelatin was further purified by electrolysis which changed it from electropositive to electronegative and reduced its conductivity.

The writer does not find that prolonged dialysis with water can be depended upon to remove such substances as the phosphates of calcium, iron and aluminum when these are originally present in the gelatin. Possibly the original gelatin used by Dhère and Gorgolewski was largely free from such compounds because of the method of its manufacture. The properties of their ash-free product, however, in some respects agree with those of the preparation to be described.

<sup>1</sup> Ostwald, *Arch. ges. Physiol.*, **108**, (1905).

<sup>2</sup> Lillie, *Am. J. Physiol.*, **20**, 127 (1907).

<sup>3</sup> Moore and Roaf, *Biochem. J.*, **2**, 34 (1906).

<sup>4</sup> Procter, *Kolloid Chem. Beihefte*, **2**, 243 (1911); *J. Chem. Soc.*, **107**, 313 (1914).

<sup>5</sup> Procter and Wilson, *J. Chem. Soc.*, **109**, 307 (1916).

<sup>6</sup> Loeb, *J. Biol. Chem.*, **31**, 343 (1917); **33**, 531 (1918); **34**, 77 (1918); **34**, 395 (1918); **34**, 489 (1918); **35**, 497 (1919); also recent articles in *Am. J. Expt. Physiol.*

<sup>7</sup> THIS JOURNAL, **41**, 135 (1919).

<sup>8</sup> Paal, *Ber.*, **25**, 1202 (1892).

<sup>9</sup> Dhère and Gorgolewski, *Compt. rend.*, **150**, 934-936 (1910).

Ash-free gelatin might be made from collagen or bone which has been sufficiently treated with acids to remove all mineral matter. Complete removal of the acid from the collagen is extremely difficult, however, and afterward it is almost impossible to change the collagen to gelatin. As a practical method for the laboratory this procedure is not promising.

A simple, rapid method, applicable to any sample of gelatin, has been worked out, whereby 30 or 40 g. of material can be prepared in a few hours. The results given in this paper were obtained entirely from such material. Unless otherwise stated, only gelatin of the highest jelly strength (maximum mutarotation ratio 2.2)<sup>1</sup> was selected. A 0.5% solution of this gelatin just gives a weak jelly at 0°; at 15° it is on the border line between a highly viscous liquid and a weak jelly.

**Method of Preparation.**—Ten g. of finely powdered gelatin (about 16 mesh) is placed in a 24 cm. folded filter supported at the tip by a cone or small funnel, and the whole in turn placed in a funnel of suitable size (15 cm.). A 10% sodium chloride solution containing about 5 cc. of conc. hydrochloric acid per liter, cooled to between 0° and 10°, is poured back and forth several times through the powdered gelatin. Fresh portions of the salt-acid mixture are then used until no test for lime can be obtained in the washings. This acid mixture is then replaced by cold 1% salt solution without acid, and washed back and forth. From now on, the concentration of the salt solutions is diminished as rapidly as control of the swelling will permit. If the salt washing were discontinued the gelatin might swell and occupy the whole filter, making washing extremely difficult. Preferably the gelatin should be permitted to swell no further than two-thirds up the filter. A considerable washing will be required with very dilute salt solutions and finally with distilled water until no chlorine is found in the washings, when the gelatin particles will have contracted to a volume of about 7 or 8 cc. per gram of gelatine. It is advisable to continue the washing with several liters of distilled, aerated-distilled, or conductivity water, if high purity is to be ensured. Finally cold 90% alcohol is poured through the drained jelly masses until they are shrunk nearly to dryness, after which they are dried with an electric fan.

**Discussion.**—When powdered gelatin is washed with cold water alone, the readily diffusible calcium salts soon pass away until further washing becomes ineffective. If it is now washed with a solution of sodium chloride, ammonium chloride, potassium bromide, or presumably any uni-univalent electrolyte, dialysis of the remaining lime salts takes place immediately, probably because certain slowly diffusible, possibly colloidal, salts of calcium react with them to form readily diffusible salts.

<sup>1</sup> THIS JOURNAL, 41, 135 (1919).

If the added electrolyte is now washed out, any alkali combined with the gelatin is almost invariably left. Using sodium chloride, sodium carbonate is found in the ash. In order to insure the removal of this alkali as well as iron, heavy metals, etc., acidulated salt solution must be used. The removal of all calcium salts can be accomplished in an hour, but the removal of the hydrochloric acid requires several hours and the use of dilute salt solution until the remaining acid can be removed by water alone without excessive swelling. It is almost impossible to wash gelatin swollen to 40 or 50 volumes. As the last traces of acid are being removed, the gelatin (at 15°) shrinks to particles swollen to about 7 volumes. The removal of the last traces of acid is probably facilitated by the fact that the iso-electric point of gelatin is on the acid side- $P_H$  4.7.<sup>1</sup>

**Properties.**—Ash-free gelatin thus obtained when incinerated leaves no ash other than traces of sand when the original glue or gelatin contains such. When ashed with pure sodium carbonate, chlorides, sulfates, or phosphates cannot be detected.

Ash-free gelatin swells in water at 15° to about 7 or 8 volumes. If such a gelatin be melted and cooled, a clear, stable jelly is produced. If, however, a weaker jelly be prepared, syneresis takes place, with the production of a cloudy jelly. A 0.5% jelly will flocculate into jelly particles (probably swollen to 7 volumes) and can be filtered off completely from the extruded water, which shows no trace of gelatin.

Ash-free gelatin forms sols or gels with a minimum tendency to remain dispersed. It is readily precipitated by alcohol without the presence of electrolytes. Traces of acids or alkalis increase the osmotic pressure and prevent its precipitation by alcohol. Gelatin thus peptized by traces of alkalis or acids in the presence of a large percentage of alcohol exhibits a marked resemblance to the metal suspensoids. Traces of electrolytes, for example those present in a drop of tap water, cause immediate precipitation. Bivalent and trivalent ions are most effective in bringing about precipitation.

Ultimate analysis of this gelatin gave the following results.<sup>2</sup>

	Carbon.	Hydrogen.	Nitrogen.	Oxygen.
	50.47	6.75	17.53	25.25
	50.56	6.87	17.53	25.04
Av.	50.52	6.81	17.53	25.15

Moisture was determined by drying at room temperature over sulfuric acid to constant weight for several weeks. Heating at 100° caused no further loss in weight. Moisture correction was applied to all figures. The carbon content was from 0.5 to 1.1% higher than that in published

<sup>1</sup> Michaelis. "Die Wasserstoffionkonzentration." Patten and Kellems, *J. Biol. Chem.*, **42**, 363 (1920).

<sup>2</sup> Carbon and hydrogen determinations were made by Dr. D. H. Brauns.

analyses made on ash-containing material, probably because the latter retained carbon or carbon dioxide which was not considered.

### Osmosis Experiments.

Thimbles of collodion having a volume of about 40 cc. were prepared by the usual methods. These were securely bound to rubber stoppers carrying glass tubes (internal diameter about 4 mm.), passing through for manometers. The pressures were measured by the vertical heights reached by the gelatin solutions and were corrected for capillarity. To lessen the resulting dilution, the solutions were placed, initially, close to the expected pressure. In measuring pressure higher than 200 mm. of water, mercury was used in a simple manometer separated from the solution by a short air space. All results, however, are expressed as millimeters of water pressure.

The same thimble should not be used for different concentrations of gelatin. In this work the most important figures were checked with freshly prepared thimbles which had previously soaked for several hours in distilled water.

When ash-free gelatin is dissolved in warm water, placed in the thimble, cooled to 10°, and immersed in water, a separation of the gelatin into jelly masses (syneresis) and extrusion of water occurs. Around 0.5% concentration the gelatin separates completely, and up to 12% very slowly, but no pressure can be detected. When a 1% jelly is cooled it soon assumes a very fine cloudiness which grows denser and denser as it contracts into jelly masses and extrudes water.

When the jellies are melted at 35°, pressures proportional to the concentration are registered. Several different samples gave approximately 48 mm. water pressure for 2 g. (1.78 dry) per 100 cc. concentration and 95 mm. for 4 g. (3.56 dry) per 100 cc. Lillie found with ordinary gelatin at 35°, 81 mm. for 1.25 g. per 100 cc. or over 2.5 times as much. Assuming the applicability of the gas laws, a molecular weight of about 96,000 is obtained.

When the gelatin solutions are surrounded with water containing alkali or acid, these diffuse in, prevent syneresis, and produce pressures. In the experiments with acids and alkalies the external solution was of comparatively large volume and in many cases was changed so that equilibrium with a definite, known concentration of external acid or alkali was obtained. Often small amounts were added in the same concentration to the gelatin solution as was used externally to lessen the amount of electrolyte which would finally diffuse in.

Only a few experiments were made with gelatin jellies in the presence of acids in collodion membranes because the pressures are not uniformly transmitted up the manometer tube. They give approximately the same

pressures as when melted. Jelly in dilute acids swollen to its "maximum," therefore, still exerts pressure when surrounded by a membrane, and tends to swell indefinitely, but when in the presence of acid without membrane it slowly disperses in the solution. This condition does not represent real osmotic equilibrium as some chemists have assumed.

The gelatin was weighed out air dry (10.7% moisture), soaked in cold water, dissolved by gentle heating, and made up in a volumetric flask to graduation mark at 35°. Aerated distilled water free from carbon dioxide was used in all experiments with alkalis and in many of those with acids.

The pressures were all noted at the end of 24 hours and considered as correct under the conditions of the experiment if less than 3 mm. change was observed at the end of another 24 hours. Often the pressures remained nearly constant for days, although at times a slow diminution occurred which might indicate leakage through the connections by passage of gelatin through the membrane.

The results in Table I show that gelatin in a concentration of 0.5 g. per 100 cc. water, at 10° in a collodion membrane in equilibrium with increasing amounts of highly ionized univalent acids, gives increasing osmotic pressures until a normality of 0.0008 to 0.001 is reached when the maximum pressure of about 158 mm. of water is obtained. Picric acid, however, gives low values, although the maximum pressure is obtained at nearly the same normality as hydrochloric acid. Picric acid causes precipitation inside the membrane when the exterior normality is 0.004. Sulfuric acid gives very low pressures, with a maximum at about the same normality as the univalent acids, but only one-third as great. Chromic acid ( $\text{CrO}_3$  in water) gives nearly the values of a univalent acid.

TABLE I.—OSMOSIS WITH STRONG ACIDS.  
Gelatin Concentration 0.5 g. per 100 cc. Temperature 10–12°.

Normality.	Pressures in mm. water								
	HCl.	HNO <sub>3</sub> .	HBr.	HI.	HClO <sub>4</sub> .	$\frac{\text{CCl}_4}{\text{COOH}}$ .	Picric.	H <sub>2</sub> SO <sub>4</sub> .	H <sub>2</sub> CrO <sub>4</sub> .
0.0001	20	...	...	...	...	...	....	..	(Molar)
.0002	70	...	...	...	...	...	....	..	...
.0004	117	...	110	...	...	107	85	..	...
.0006	132	...	...	...	...	...	....	43	84
.0008	156	...	153	...	...	154	109	..	...
.0010	156	152	150	157	160	154	98	48	107
.0015	154	...	...	...	...	...	....	..	119
.0020	133	129	128	...	127	128	50	40	90
.0030	113	103	108	...	97	107	14	25	79
.0040	90	81	75	78	79	79	Ppt.	..	56
.0070	53	...	...	...	...	...	Ppt.	..	...
.0090	37	...	...	...	...	...	....	..	...

Special precautions were taken in obtaining the results at a normality of 0.004, at which concentration nitric, hydrobromic, hydriodic, per-

chloric, and trichloro-acetic acids give almost identical pressures. Hydrochloric acid appears to give a little higher pressure.

The results in Table II show that phosphoric, acetic, malic, salicylic, formic and lactic acids give the same maximum pressure of about 158 mm., which was found with the highly ionized univalent acids, but at different molar concentrations. All of these acids are known to dissociate almost wholly into univalent anions.

TABLE II.—OSMOSIS WITH WEAK ACIDS.  
Gelatin concentration 0.5 g. per 100 cc. Temperature 10–12°.

Molar.	Pressures in mm. water						
	H <sub>3</sub> PO <sub>4</sub> .	Oxalic.	Salicylic.	Malic.	Formic.	Lactic.	Acetic.
0.001	129	123	...	123	97	...	...
.0015	159	129	...	133	...	...	...
.0020	159	119	150	141	132	140	...
.003	139	110	154	147	150	...	71
.004	125	...	132	149	157	159	...
.005	...	...	126	...	...	...	...
.006	...	...	...	...	159	160	...
.008	...	...	...	...	153	161	106
.010	...	...	...	...	153	156	113
.100	...	...	...	...	75	78	156
1.000	...	...	...	...	...	...	79

Calculating the values of  $v$  from the formula  $10^{-6v}/(1 - 10^{-3v}) = k$ , where  $k$  is the ionization constant of the acid, the following values are obtained: 910 for phosphoric (molar), 980 for oxalic (molar), 285 for malic (molar), 176 for formic, 123 for lactic, and 18 for acetic. These values represent the dilutions at which the hydrogen-ion concentration is  $1 \times 10^{-3}$ . In every case the maximum pressure falls about the concentration indicated, just as with the highly ionized univalent acids.

Sulfuric acid is known to dissociate as a bivalent acid, ionizing almost completely as  $H_2^{++} + SO_4^{--}$  at these dilutions. This offers a plausible explanation of its abnormal behavior. Chromic acid ( $CrO_3$  in water) is known to ionize principally into univalent ions,  $H^+ + HCrO_4^-$ , but also into some  $H_2^{++} + CrO_4^{--}$  and possibly  $H_2^{++} + Cr_2O_7^{--}$ . These facts are consistent with the intermediate values given. Oxalic acid gives a maximum of only 130 mm. pressure which similarly indicates some dissociation into a bivalent anion in harmony with the known dissociation of its second hydrogen ion. Picric acid presents an exception to this reasoning.

The results in Table III show that alkalis produce a maximum at a normality of  $2 \times 10^{-4}$ . The univalent bases give a value of about 165 mm. pressure, while the bivalent bases give a value of 54. It appears to be more than a coincidence that univalent acids or bases give nearly the same maximum pressure and bivalent acids or bases (ionizing as such) also give nearly the same.

TABLE III.—OSMOSIS WITH ALKALIES.  
Gelatin Concentration 0.5 g. per 100 cc. Temperature 10–12°.

Normality.	Pressures in mm. water						
	NaOH.	KOH.	LiOH.	NH <sub>4</sub> OH.	C <sub>5</sub> H <sub>11</sub> N.	Ca(OH) <sub>2</sub> .	Ba(OH) <sub>2</sub> .
0.0001	119	...	...	...	...	...	50
.0002	169	160	161	...	...	54	54
.0003	148	151	...	...	...	48	48
.0004	146	...	...	...	...	...	...
.0005	...	135	...	...	171	42	43
.0008	136	...	...	...	...	...	...
.0010	110	...	...	164	142	...	...
.0020	86	...	...	171	122	...	...
.0030	...	...	...	163	114	...	...
.0050	...	...	...	153	94	...	...
.0080	...	...	...	143	...	...	...
.1000	...	...	...	79	...	...	...

$1 \times 10^{-5}$  NaOH gave 29 mm.;  $3 \times 10^{-5}$ , 48 mm.;  $5 \times 10^{-5}$ , 65 mm.

Ammonium hydroxide acts in the same manner as the strong monovalent alkalis, but requires a greater normality to reach the same hydroxylion concentration, as is to be expected.

Piperidine (C<sub>5</sub>H<sub>11</sub>N) is a strong base with  $k$  equal to  $1.0 \times 10^{-3}$ . It gives results to be expected from a univalent base.

Experiments with a saturated solution of silver hydroxide not recorded indicate that if it were more soluble it would behave strictly as a univalent base.

The general results reported in Tables I, II, III are represented graphically in Figure 1.

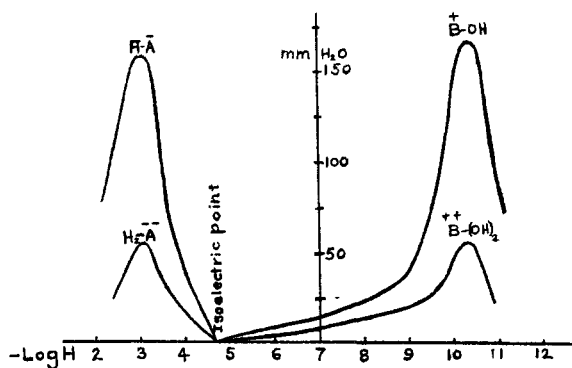


Fig. 1.—Osmosis of gelatin with bases and acids at 10°;  
0.5 g. gelatin per 100 cc. H<sub>2</sub>O.

The results in Table IV show that the normality of acid giving maximum pressure increases with increasing concentration of gelatin and in about the same ratio. Increasing temperature and dilution probably favor the decomposition of the combined gelatin and acid, while the proportion

of uncombined repressing ions is increased. The normality of acid for maximum pressure at 35° is about  $0.8 \times 10^{-3}$  for 0.5 g. per 100 cc., while for 2 g. per 100 cc. it is at least  $3.0 \times 10^{-3}$ . This point bears on the normality of the swelling of gelatin powder or plates to be further emphasized.

TABLE IV.—EFFECT OF TEMPERATURE AND CONCENTRATION OF GELATIN ON OSMOSIS WITH HYDROCHLORIC ACID.

Normality.	Quantities in g. per 100 cc.			Pressures in mm. water		
	0.2 g. at 10°.	0.4 g. at 10°.	0.5 g. at 10°.	0.5 g. at 35°.	1.0 g. at 35°.	2 g. at 35°.
0.0002	..	52	70	95	...	....
.0004	37	95	117	139	...	....
.0006	..	...	132	...	...	....
.0008	42	...	...	189	489	....
.0010	41	108	156	180	...	1135
.0015	34	103	154	177	557	....
.0020	24	81	133	166	557	1360
.0030	22	79	113	...	489	1455
.0040	20	60	...	...	428	....
.0050	..	46	...	...	380	....
.0080	..	...	...	...	285	....

The results in Table V show clearly the great depression produced by solutions of salts with the common ion of the acid or alkali employed. The fact that barium chloride gives the same effect as sodium chloride in hydrochloric acid osmosis when the chlorine ion concentration is the same is noteworthy. Perchloric acid and sodium perchlorate act quantitatively (results not given in the table) in the same manner as do hydrochloric acid and sodium chloride. It is probable that all similar highly ionized univalent acids and their salts would give the same values.

TABLE V.—EFFECT OF SALTS ON ACID AND ALKALI OSMOSIS.

	Gelatin 0.5 g. per 100 cc.		Temperature 10–12°.	
	Normality.	Salt.	Normality.	Pressure.
Hydrochloric	0.001	.....	0	156
	.001	NaCl	0.001	103
	.001	BaCl <sub>2</sub>	.001	103
	.001	NaCl	.002	69
	.001	BaCl <sub>2</sub>	.002	70
	.001	NaCl	.005	39
Formic	.004	NaFor.	0	157
	.004		0.001	71
	.004		.002	44
Lactic	.004	Na Lac.	0	159
	.004		0.001	90
	.004		.002	37
			0	146
NaOH	.0003		0	146
	.0003	NaCl	0.0005	69
	.0003	NaCl	0010	47



Only a few experiments have been made on mixtures of acids or bases with or without their respective salts but it is to be expected that the pressures obtained will be dependent solely on the total hydrogen or hydroxyl-ion concentration together with the respective effects of all the anions or cations present.

Solutions of citric acid and sodium hydrate and those of disodium citrate and hydrochloric acid prepared to give a hydrogen-ion concentration  $1 \times 10^{-3}$  gave only 7 mm. and 10 mm. pressures, respectively. These extremely low pressures probably result from the excessive amounts of univalent and possibly bivalent anions present.

#### Absorption of Acids and Alkalies and the Swelling of Gelatin.

When one gram of air-dry gelatin is immersed in water at  $15^{\circ}$  it swells to a volume of 7 or 8 cc.; when immersed in the proper concentration of certain acids or alkalies, it may swell to as much as 48 and 30 cc., respectively. If a limited volume of the acid or alkali solution is used, the unabsorbed or external solution becomes diminished in concentration. Assuming that the free acid or alkali in the jelly is of the same concentration as in the external solution, the total uncombined may be calculated. This, subtracted from the total, gives the combined portion.

In the experiments listed below, 100 cc. of the solution to be studied was poured over 1 g. of air-dry (10.7% moisture) powdered gelatin contained in an Erlenmeyer flask or a 100-cc. graduated cylinder. Equilibrium is reached readily in two hours in the flask, and the more accurate absorption data are thus obtained, while the cylinder requires about 4 hours, with frequent agitation, and gives the swelling volume in addition. Before reading the volume of the jelly particles they were allowed to settle for several hours, or, more often, overnight. While the swelling of blocks of gelatin might give more accurate data, the results with powdered gelatin are quickly obtained and are considered sufficiently accurate for the conclusions derived. The jelly particles when much swollen pack together closely, and it has been found that this volume differs less than 2% from the swelling of a single block. When ash-free gelatin is immersed in dilute sodium chloride or potassium bromide solution, no diminution in concentration can be detected. It is doubtful whether any appreciable absorption or combination takes place with salt electrolytes, at least in 0.1 *N* solutions or weaker.

The results in Table VI show that hydrochloric, nitric, hydrobromic, thiocyanic, perchloric, and hydriodic acids are absorbed in exactly the same manner by gelatin and produce the maximum swelling when the external acid is 0.004 *N*. If experiments were made simultaneously with all of these acids under exactly the same conditions the amounts absorbed doubtless would be within the experimental titration error.

TABLE VI.—ABSORPTION AND SWELLING OF GELATIN WITH ACIDS.

Total. Cc.	Free. Cc.	Combined. Cc.	Jelly. Volume. Cc.
0.1 N Hydrochloric acid.			
0.98	0.36	0.62	17.5
1.97	0.60	1.37	22.5
2.95	0.91	2.04	29.0
3.93	1.20	2.73	34.
9.84	4.50	5.34	42.
14.76	8.80	5.96	....
19.68	13.56	6.12	34.
19.68	13.40	6.28	....
24.10	18.50	6.10	....
Hydrobromic acid.			
4.84	1.56	3.28	40.
9.68	4.34	5.34	46.
19.36	13.34	6.02	38.
29.04	23.06	5.98	31.5
Picric acid.			
5.56	1.50	4.06	7
11.12	4.10	7.02	16
Nitric acid.			
14.93	9.26	5.67	....
19.90	13.84	6.06	....
24.87	18.84	6.03	....
34.82	28.56	6.24	....
Thiocyanic acid.			
9.54	4.02	5.52	41
15.90	9.66	6.24	39
31.23	24.84	6.39	30
Trichloro-acetic acid.			
3.98	1.60	2.38	28.5
9.95	4.40	5.55	45.0
19.90	13.80	6.10	37.5
29.85	23.80	6.05	31.5
Perchloric acid.			
5.0	1.44	3.56	44.5
10.0	4.40	5.60	48.5
15.0	8.90	6.10	45.0
20.0	13.94	6.06	41.0
Hydriodic acid.			
5.0	1.60	3.40	36.
10.0	4.46	5.54	47.
15.0	8.90	6.10	43.
20.0	14.20	5.80	39.
Oxalic acid. 0.1 M.			
4.97	1.77	3.20	33.
9.95	5.00	4.50	42.
14.92	9.20	5.72	41.
24.87	18.87	6.00	....

TABLE VI.—ABSORPTION AND SWELLING OF GELATIN WITH ACIDS—(Continued.)

Acetic acid. 0.1 <i>N</i> .			
Total. Cc.	Free. Cc.	Combined. Cc.	Jelly. Volume. Cc.
20.10	18.90	1.20	....
Sulfuric acid.			
9.76	3.56	6.20	25
14.64	7.88	6.76	26
19.52	12.96	6.56	24
Phosphoric acid. 0.1 <i>M</i> .			
5.0	2.15	2.85	43
10.0	5.42	4.57	49
15.0	9.40	5.60	51
25.0	19.50	5.50	48
50.0	44.50	5.50	40
Chromic acid. 0.1 <i>M</i> .			
5.0	1.15	3.85	25
10.0	3.35	6.65	24
15.0	7.35	7.65	20
20.0	11.65	8.32	15

Sulfuric acid produces much less swelling than the univalent acids, and a little more combines with the gelatin. Picric acid is a precipitant of gelatin, but small amounts produce some swelling and the absorption is greater than with even sulfuric acid.

With polybasic acids ionizing with a univalent anion like phosphoric or oxalic acid (chiefly) the absorption as  $H^+ + H_2PO_4^-$  or  $H^+ + HC_2O_4^-$ , respectively, agrees with the absorption of the univalent acids, perchloric, hydrobromic, etc., confirming the results on osmosis, according to which these acids gave the same pressure as univalent acids. The quantity absorbed appears to depend on the hydrogen-ion concentration (calculated from conductivity data).

Chromic acid gives abnormal results in absorption and swelling, due possibly to the presence of ions such as  $Cr_2O_7$ , etc.

The results in Table VII show that alkalies do not give a marked maximum of swelling. Marked solution or dispersion as observed by a frothing tendency is indicated. Univalent bases give greater swelling than bivalent, but probably not in the ratio that might be expected from the osmotic pressures. This probably is due to the relatively greater solution effects. It should be noted that bivalent bases give about the same swelling maximum as bivalent sulfuric acid. Increased absorption is obtained with the bivalent bases that give less swelling.

Using 10 cc. of 0.1 *N* hydrochloric acid per 100 cc. with gelatin at 0° and 10°, volumes of 34 cc. and 44 cc., respectively, were obtained. Ash-free, low jelly strength gelatins give decreased swelling. Doubtless these results are due to the fact that the cohesion of the jelly is diminished. It

is probable that ash-free gelatins at low temperatures would give volumes nearly proportional to their jelly strength, which is not true of those with varying ash contents.

TABLE VII.—ABSORPTION AND SWELLING OF GELATIN WITH ALKALIES.

Total. Cc.	Sodium hydroxide. 0.1 N.		Volume. Cc.
	Free. Cc.	Combined. Cc.	
1.0	0.34	0.66	18
2.0	0.66	1.34	22
3.0	0.76	2.24	26
4.0	1.36	2.64	30
5.0	2.12	2.88	30
7.0	3.60	3.40	30
10.0	6.00	4.00	27
20.0	15.60	4.40	26
	Calcium hydroxide.		
14.81	9.20	5.61	23
	Potassium hydroxide.		
5.0	1.60	3.40	..
10.0	5.50	4.40	..
	Barium hydroxide.		
5.0	1.10	3.90	23
10.0	4.80	5.20	23
15.0	8.84	6.16	23
20.0	14.20	5.80	22

The results in Table VIII show that sodium chloride increases the absorption of hydrochloric acid by gelatin. Any assumption that the free acid in the jelly occurs in lower concentrations than the external acid could not vitiate that conclusion. Similarly, sodium chloride increases the absorption of alkali.

TABLE VIII.—EFFECT OF SALTS ON THE SWELLING AND ABSORPTION OF ACIDS AND ALKALIES BY GELATIN.

	0.1 N Acid. Cc.	Salt.	0.1N Salt. Cc.	Free. Cc.	Combine. Cc.	Volume. Cc.
HCl	5.00	None	.....	1.56	3.44	37
	5.00	NaCl	5	0.80	4.20	31
	5.00	NaCl	20	0.50	4.50	25
	5.00		100	0.34	4.66	18.5
	9.84	None	None	4.50	5.34	42.
	9.84	NaCl	20	3.26	6.58	29.5
	19.68	None	None	13.56	6.12	34.
	19.68	NaCl	20	12.70	6.98	30.
	19.68	NaCl	80	12.20	7.48	23.
NaOH	20.00	NaCl	None	15.60	4.40	....
	20.00	NaCl	40	13.34	6.66	....

### Theories of Osmosis and Swelling.

Lillie explained the increased osmotic pressure of gelatin in the presence of dilute acids as due to the breaking down of molecular aggregates to

smaller and hence more numerous particles. As Lillie neither extended his work to the diminution of the pressure on increasing the concentration of the acid, nor studied the similar phenomena of alkali osmosis, or the effect of salt ions on acid and alkali osmosis, his conclusions were based on insufficient data.

Procter, and Procter and Wilson made a very exhaustive study of the swelling of gelatin in hydrochloric acid. Procter at first determined the absorption of acid by titrating the drained and melted jelly with alkali, using phenolphthalein and methyl orange for indicators. Later Procter abandoned the direct titration method, substituting for it the addition of salt to shrink the jelly, and titrating the salt solution to obtain the "internal" uncombined acid. Knowing the external acid, and the uncombined acid of the jelly, the sum of these taken from the total used gave the combined acid.

Procter first held the view that salt lessened the hydrolysis of the gelatin chloride by depressing its ionization, but Procter and Wilson later ignored this factor. If salt increases the absorption of hydrochloric acid by the gelatin, their method of obtaining the internal uncombined acid is incorrect. The writer made the experiments reported in Table VIII to test this point specifically, and found that salt unquestionably increases the absorbed acid. Tolman and Stearn<sup>1</sup> also have stated that salt increases the absorption of acid by fibrin jellies. They have further assumed the same concentration of the hydrogen within and without the jelly.

In the most recent work of Procter and Wilson<sup>2</sup> and Wilson and Wilson<sup>3</sup> the force which causes the swelling of gelatin jellies is ascribed to the excess of diffusible ions in the jelly over those of the external solution. The following equations express their conception of the factors involved.

$H^+{}_x + Cl^-{}_x \rightleftharpoons H^+{}_y + Cl^-{}_y$ ,  $G^+{}_z + Cl^-{}_z$ ,  $G\ Cl$  (jelly phase), and therefore

$$H^+{}_x \times Cl^-{}_x = H^+{}_y(Cl^-{}_y + Cl^-{}_z) = K(HCl),$$

or (1)  $x^2 = y(y + z).$

It will be noted that a different hydrogen-ion concentration is assumed in both phases, but it is assumed that the theoretically small quantity of undissociated hydrochloric acid is uniformly distributed between external acid and the jelly.

The excess of diffusible ions in jelly phase is represented by (2)  $y + y + z - 2x$ , called  $e$ , to which Procter and Wilson ascribe the force causing swelling. From Equations 1 and 2 they algebraically derive  $e = -2x + \sqrt{4x^2 + z^2}$ .

Procter and Wilson produce no direct experimental evidence to show that

<sup>1</sup> Tolman and Stearn, *THIS JOURNAL*, 40, 264 (1918).

<sup>2</sup> Procter and Wilson, *J. Chem. Soc.*, 109, 307 (1916).

<sup>3</sup> Wilson and Wilson, *THIS JOURNAL*, 40, 264 (1918).

the hydrogen-ion concentration of the jelly is always less than that in the external acid solution, as their theory assumes. When the jelly is liquefied they assume that this  $\epsilon$  is transferred from around the jelly mass to immediately around the gelatin particles, and the bulk of the acid solution becomes the external phase. Gelatin at  $10^\circ$  in 0.4% concentration is a viscous liquid; in 0.6% concentration it is a jelly. When does the external phase change its location from around the particles to around a jelly mass? Moreover, it is difficult to understand how the chlorine ions attached to gelatin decrease the hydrogen ions of the hydrochloric acid, when it is known that dilute salt affects it but slightly, or even increases the hydrogen-ion concentration. In settling this point indicators are useless in the presence of such an ampholyte as gelatin because they combine with the gelatin as a base or acid, and hydrogen electrode experiments are not possible in the jelly.

Loeb treated gelatin with sodium chloride, ammonium chloride, and other salts, then washed away the excess of electrolyte and obtained increased swelling. Loeb overlooked the real explanation of his experiments. These electrolytes remove the repressing lime salts and leave a gelatin combined with sodium cations. Loeb found that bleached gelatin failed to give the same result, probably because less alkali was left. The ash of these treated gelatins consists of sodium carbonate. The writer had prepared his ash-free gelatin before the work of Loeb had come to his attention when the meaning was apparent. It is not surprising that calcium, magnesium, strontium, barium chloride, or magnesium sulfate produced no increased swelling, for they do not remove the ash, and they also leave combined bivalent cations which do not increase swelling as much as univalent cations.

Loeb continued to treat gelatin with various salts, under the impression that they were reacting with the gelatin. Only when using oxalates does he mention the formation of a white precipitate (obviously from the lime). He (Jan. 20, 1919) ascribes the increase in osmotic pressure of gelatin in the presence of acids to an increase in the number of particles, ionization not considered, but later stated that free hydrobromic acid represses the ionization of gelatin bromide and again that the physical properties of gelatin are dependent only on the number of gelatin bromide molecules formed. The writer takes exception to the titration of gelatin in the presence of hydrobromic acid as giving nearly 90% of the acid combined with 10% of free acid. The results on the absorption of acids by gelatin show a ratio of combined 2 to 1 in strong jellies, but in 1% solution the hydrolysis is much greater and the free acid may be equal at least to the combined acid. This error further leads Loeb to ignore the repression of the ionization of the gelatin bromide by the free dissociated hydrobromic acid. Loeb's figures for osmotic pressure obtained on incompletely

purified gelatin are from 25 to 50% too low. The results of this paper, however, confirm many of his conclusions.

Coming to the correlation of the osmosis experiments with those on swelling, it can be said that swelling in general is a reflection of the osmotic pressure found in the collodion membrane. Undoubtedly this is not the only factor. The osmotic pressure maximum of alkalies is the same as that of acids, while the swelling is little more than three-fifths as much.

The gelatin acts as its own membrane in resisting the osmotic pressure of the combined ions or the repulsion of the gelatin particles bearing the same charge (if the latter theory is adopted). Can we expect it to do so indefinitely? It is probable that gelatin swells until the leakage of the membrane is such that the cohesion of the particles equals the osmotic pressure minus leakage. Swollen gelatin is therefore in the process of slow dissolution and does not represent equilibrium between osmotic pressure and cohesion alone. The writer's experiments show that the hydrogen-ion concentration of maximum osmotic pressure varies with the concentration of the gelatin. The maximum for 0.5 g. per 100 cc. is about  $P_H$  3.0 to  $P_H$  3.2, corresponding to approximately 0.001 *N* highly ionized acid, but for 2 g. per 100 cc. it is about 0.004 *N*, in agreement with the normality of acid giving the swelling maximum of jellies swollen to 50 volumes (about 2 g. per 100 cc.).

The osmotic pressure of gelatin in its sols and gels increases with increasing concentration of acids or alkalies, because greater combination results. The uncombined ionized acid probably from the very first diminishes the pressure. After the "maximum" is reached the added anions of the acid or cations of the base more than offset the increased combination, and the pressures diminish. Increasing temperature favors hydrolysis of the compound, so that the repression is reached at a lower concentration. Increasing concentrations of the gelatin favor greater combination with the acid and less proportional depression. The osmotic pressure increases more rapidly than the concentration of gelatin.

Experiments with ash-free gelatins of low jelling power give the same osmotic pressure as those of the highest power but swell much less, the swelling being nearly proportional to jelling power. It seems probable that all swell to a limiting cohesion. The slight differences in swelling with different acids of the same valence may result from their varying effects on the cohesion. If a honeycomb structure for gelatin jelly is assumed, the rapid swelling of dry gelatin in dil. acids or alkalies can be explained as resulting from the combined swelling of all the cell units. At the "maximum" of swelling, it still exerts considerable pressure as shown when confined in a collodion membrane, and, if additional space is

offered, will slowly occupy the whole space as a jelly by a process of dissolution.

The experiments do not decide whether the pressure results from the electric charge of the gelatin particles or from the equal but opposite charge of the attached ions. It appears incorrect to consider a lump of jelly or the liquid contents of a collodion sac as a phase distinct from the surrounding liquid. The bulk of the enmeshed liquid in the jelly or sac probably has the same concentration of electrolytes as the exterior liquid. The magnitude of the osmotic pressure (if it be correct to call it such) is conditioned by the electric charges of the gelatin molecules (or particles) and the ionic field immediately surrounding.

The results of this paper are quantitatively reproducible. As the study of the properties of gelatin has advanced, little excuse is seen for applying the term "hysteresis" to the failure to reproduce conditions and results.

#### Summary.

1. Gelatin free from electrolytes (ash-free) has been prepared and used in the experimental work.

2. The osmotic pressure of this gelatin in the presence of water is proportional to concentration, and gives a molecular weight of 96,000, assuming the applicability of the gas laws.

3. Gelatin sols (probably also gels) in a collodion membrane immersed in isohydric solutions of univalent acids, or polyvalent acids ionizing as a univalent acid produce the same osmotic pressure. Similarly, when immersed in univalent bases the same osmotic pressure is produced at the same hydroxyl-ion concentration.

4. Minimum osmotic pressure is produced at the iso-electric point where it is not combined with acids or alkalies. With increasing hydrogen- or hydroxyl-ion concentration the osmotic pressure increases to a maximum and then diminishes.

5. The maximum osmotic pressure for 0.5 g. of gelatin per 100 cc. at  $10^{\circ}$  in univalent acids or polyvalent acids ionizing as univalent, is reached at a hydrogen-ion concentration of 0.8 to  $1.0 \times 10^{-3}$ , and amounts to about 158 mm. of water. In univalent bases the maximum is reached at  $0.2 \times 10^{-3}$  hydroxyl-ion concentration and amounts to about 165 mm.

6. Using bivalent acids or bases ionizing as such, the maximum osmotic pressure is reached at the same hydrogen- or hydroxyl-ion concentration as with univalent acids or bases, respectively, but amounts to about 55 mm., or about one-third as much.

7. When dry gelatin is immersed in univalent acids, or polyvalent acids ionizing as univalent, it combines with equivalent amounts at the same hydrogen-ion concentration. The amount of swelling is approxi-



mately the same for all, its maximum occurring at about  $4 \times 10^{-3}$  hydrogen-ion concentration when one gram of air-dry gelatin occupies a volume of about 46 cc. Bivalent ionizing acids give much less swelling with a maximum at about the same point, and combine in somewhat greater equivalent amounts.

8. Salt ions do not combine with gelatin, but increase the absorption of alkalis or acids. They markedly decrease swelling and osmotic pressure, probably by decreasing the ionization of acids or alkalis combined with the gelatin. Since salt ions do not appreciably affect the ionization of the dilute highly ionized mineral acids or bases, the hydrogen or hydroxyl ion is not the determining factor when salts are present. The action of buffer mixtures likewise is not determined by the hydrogen-ion concentration.

9. The swelling of gelatin is the result of osmotic pressure within the jelly, with the jelly acting as an imperfectly resisting membrane, the more so when highly swollen. While the osmotic pressure at the optimum concentration of univalent acids and bases is the same, the swelling is much less in alkalis because of the weakened membrane effect. Bivalent sulfuric acid gives the same swelling as bivalent calcium or barium hydroxide when swelling is small and the solution is not so great.

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## SPIRO-PYRIMIDINES. II. CYCLOHEXANE-1,5-SPIRO-PYRIMIDINES.

BY ARTHUR W. DOX AND LESTER YODER.

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In the first paper<sup>1</sup> of this series a number of derivatives of cyclobutane-1,5-*spiro*-pyrimidine were described. They may be regarded as variously substituted barbituric acids in which the 5-carbon atom enters into a 4-membered hydrocarbon ring. The method of preparation consists in condensing an  $\alpha, \omega$ -dibromo-paraffin with ethyl malonate with formation of a cycloparaffin-1,1-dicarboxylic ester; the second ring is closed by condensation of the two carbethoxyl groups with urea according to the well-known veronal synthesis. By this method it should be possible also to obtain other *spiro*-pyrimidines containing 5- and 6-membered hydrocarbon rings. Starting, for example, with 1,5-dibromopentane instead of 1,3-dibromopropane, the above method of synthesis should yield a cyclohexane-1,5-*spiro*-pyrimidine.

<sup>1</sup> Dox and Yoder, THIS JOURNAL, 43, 677 (1921).